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Type I Interferon Contributes to CD4$^+$ T Cell Depletion Induced by Infection with HIV-1 in the Human Thymus$^{\nabla}$

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Persistent induction of type 1 interferon (IFN) is associated with human immunodeficiency virus type 1 (HIV-1) infection. We report here that the pathogenic HIV strain R3A (HIV-R3A) induced high levels of type 1 IFN, while the nonpathogenic HIV-R3B showed no significant induction in human fetal thymus organ culture (HFTOC). We found that IFN contributed to the depletion of human T cells by HIV-R3A in a fusion-independent fashion. The R3B recombinant with the R3A Env V1V2 domain (R3B/A-V1V2) was able to induce type 1 IFN, which contributed to the increased depletion of T cells. Therefore, type 1 IFN induction plays a significant role in HIV-induced T cell depletion in the human thymus.

Pathogenic infections of humans and rhesus macaques by human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), respectively, are characterized by generalized immune activation and progressive CD4 T cell depletion (3, 23). It has been proposed that chronic activation of interferon (IFN) production may play a role in CD4 T cell depletion and AIDS progression (1, 5, 8–10). Although *in vitro* studies have demonstrated that type 1 IFN can suppress HIV type 1 (HIV-1) viral replication, several *in vivo* studies and clinical trials have engendered mixed results in the efficacy of alpha IFN (IFN-α) treatment and control of HIV-1 (19–21). This is further complicated by recent reports that IFN-α produced by plasmacytoid dendritic cells (pDC) may mediate CD4 T cell depletion (5–7). The study of the role of type 1 IFN in HIV-1 pathogenesis is greatly limited by the lack of a relevant experimental model for HIV infection and pathogenesis. We have shown that human fetal thymus organ culture (HFTOC) closely models HIV infection and pathogenesis *in vivo* (15) in terms of viral replication and CD4$^+$ T cell depletion (2, 13, 16, 18). In addition, pathogenic HIV-1 infection in HFTOC is associated with IFN induction (4, 11, 12).

HIV strain R3A (HIV-R3A) (but not HIV-R3B) is highly pathogenic in the human fetal thymus organ culture (HFTOC) model or in SCID-hu Thy/Liv mice in *vivo* (14, 15, 17, 22). When type 1 interferon (IFN) was measured, IFN was highly induced in HFTOC infected with R3A but not with R3B (Fig. 1A). When human IFN-α/β were neutralized with a specific neutralizing antibody (nAb), almost all the type 1 IFN was blocked (Fig. 1B). We evaluated the role of type 1 IFN-α/β in HIV-R3A-mediated pathogenesis in HFTOC. Consistent with the antiviral activity of type 1 IFN, neutralization of IFN-α/β with the neutralizing antibody (nAb) significantly enhanced HIV-1 replication in HFTOC (data not shown and see Fig. 3). Interestingly, blocking IFN-α/β with nAb alone only slightly prevented HIV-R3A-mediated T cell depletion. Furthermore,

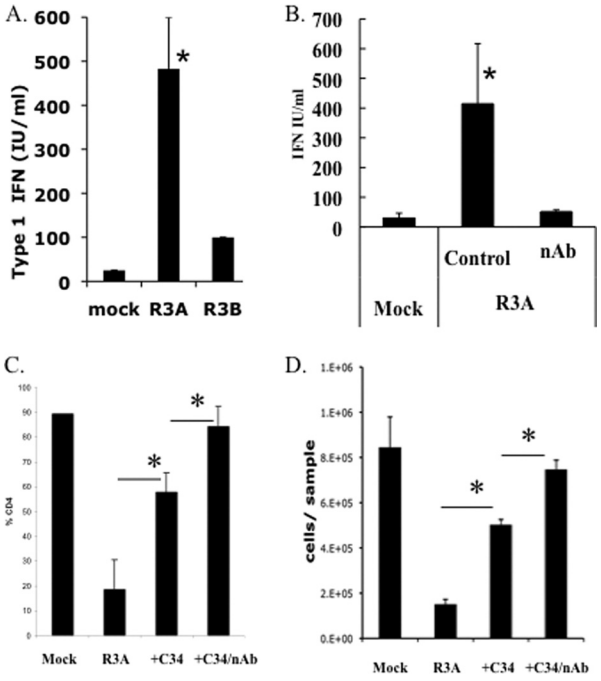

FIG. 1. HIV-R3A preferentially induces type I IFN that significantly contributes to CD4 T cell depletion. (A) Type 1 IFN induction was measured in HFTOC infected with HIV-R3A and HIV-R3B. Supernatants were harvested 24 h after infection for detection by type 1 IFN bioassay. (B) HFTOC was infected with HIV-R3A, and supernatant was treated with control rabbit IgG antibody or rabbit anti-human IFN-αβ neutralizing antibodies (nAb) during the IFN bioassay (neutralizing antibodies against human IFN-α and -β were obtained from the Biodefense and Emerging Infections Resources Repository, BEI Resources). (C, D) HFTOC was infected with HIV-R3A in the presence or absence of IFN-α/β neutralizing antibodies (nAb), fusion inhibitor C34, or both IFN-α/β nAb and C34. IFN nAb (neutralizing antibodies against human IFN-α and -β were obtained from the Biodefense and Emerging Infections Resources Repository, BEI Resources) or control antibody was added to HIV-R3A alone at 0 days postinfection (dpi), and the fusion inhibitor C34 was added at 5 dpi. HFTOC was harvested at 8 dpi to measure CD4 thymocyte depletion by the percentage of CD4$^+$ T cells (C) or the total number of CD4 T cells per HFTOC fragment. Error bars indicate standard deviations ($n = 3$). *, $P < 0.05$.

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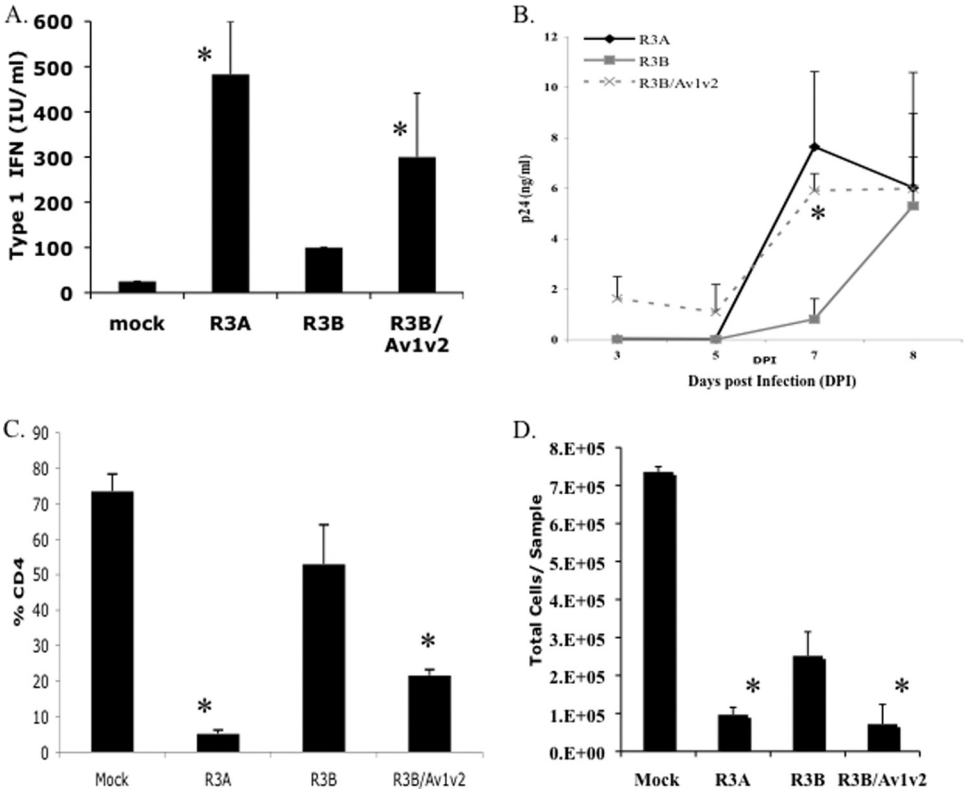

FIG. 2. The R3A V1V2 domain confers both enhanced IFN induction and pathogenesis in HFTOC. (A) HFTOC fragments were infected with R3A, R3B, and the R3B/A-V1V2 chimera recombinant. Supernatants were harvested at 24 h postinfection and analyzed by IFN bioassay. Error bars indicate standard deviations ($n = 3$). (B, C) HFTOC was infected with R3A, R3B, and the R3B/A-V1V2 recombinant. Viral replication (B) was measured by p24 enzyme-linked immunosorbent assay (ELISA), and $CD4^+$ thymocyte depletion (C) was measured by FACS analysis at 8 dpi. Error bars indicate standard deviations. *, $P < 0.05$.

when HIV-mediated fusion was also inhibited with the fusion inhibitor C34 during peak viral replication, IFN nAb further significantly rescued human thymocytes (Fig. 1C and D). We conclude that induction of IFN-α/β by HIV-R3A infection contributes to its highly pathogenic activity, at least partly via a fusion-independent mechanism of CD4 T cell killing.

We mapped the R3A Env domains that contributed to the induction of type 1 IFN with Env recombinants between R3A and R3B. Although the R3B recombinant with the R3A Env V1V2 domain (R3B/A-V1V2) with a defective Nef gene showed pathogenic activity similar to that of HIV-R3B (14, 15, 17, 22), we studied the relative pathogenicity of the R3B/A-V1V2 recombinant in comparison to R3A and R3B when their Nef genes were restored. When tested in the HFTOC model, the R3B/A-V1V2 recombinant demonstrated higher IFN induction activity than HIV-R3B (Fig. 2A). Interestingly, R3B/A-V1V2 also showed significantly higher replication than HIV-R3B in HFTOC, even though it induced higher levels of IFN (Fig. 2B). When human CD4 thymocyte depletion was measured, R3B/A-V1V2 also showed elevated pathogenic activity compared to that of R3B (Fig. 2C and D). Therefore, the V1V2 domain of R3A is one of the R3A pathogenic determinants in the presence of a functional Nef gene. Since R3B/A-V1V2 still showed lower pathogenic activity than R3A, R3A Env encodes additional pathogenic determinants, such as the high fusion activity (17, 22).

We reasoned that the pathogenic activity of the R3B/A-V1V2 recombinant should be more dependent on the type I IFN activity. We thus evaluated the role of type 1 IFN in HIV-R3B/A-V1V2-mediated pathogenesis in HFTOC by blocking IFN with a neutralizing antibody (nAb). Consistent with the antiviral activity of type 1 IFN, neutralization of IFN significantly enhanced HIV-1 replication in HFTOC (Fig. 3A). Interestingly, blockage of IFN almost completely prevented R3B/A-V1V2-mediated T cell depletion even with elevated HIV-1 replication (Fig. 3B). With a weak fusogenic activity of R3B Env, fusion inhibitor C34 did not show a significant protective effect on CD4 T cell depletion by R3B/A-V1V2. We conclude that induction of type 1 IFN by the R3A-V1V2 domain contributes to its high pathogenic activity (14).

We have previously reported that Env-mediated fusion plays a major role in $CD4^+$ depletion during infection of HFTOC with HIV-R3A (17). We found here that R3A-induced IFN-α/β led to fusion-independent CD4 T cell depletion. IFN neutralizing antibodies significantly inhibited CD4 T cell depletion, even in the presence of higher levels of HIV-1 replication. Thus, type 1 IFN that provides an antiviral response also contributes to the depletion of CD4 T cells by HIV-1 infection. In HFTOC infected with the R3B/A-V1V2 recombinant, which encodes the weak R3B fusion activity but the strong IFN induction of R3A-V1V2, type 1 IFN seemed to be the major determinant of CD4 depletion. We conclude that the patho-

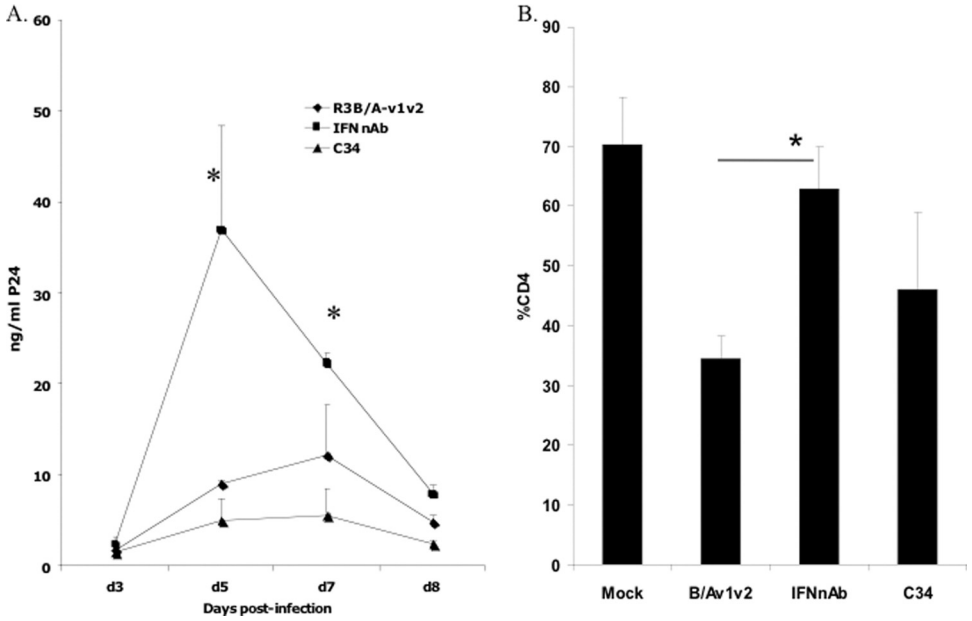

FIG. 3. IFN-α plays a critical role in R3B/A-V1V2-mediated CD4 T cell depletion in HFTOC. HFTOC was infected with R3B/A-V1V2 in the presence of IFN-αβ nAb or C34. IFN nAb was added at 0 dpi, while C34 was added at 5 dpi, as described for Fig. 1B. (A) IFN nAb enhanced HIV-1 replication in HFTOC. HIV-1 replication was measured in HFTOC supernatant by p24 ELISA. (B, C) IFN nAb inhibited R3B/A-V1V2-mediated CD4 T cell depletion. HFTOC was harvested at 9 dpi, and CD4 T cell depletion was measured by the percentage of $CD4^+$ cells and by the total number of CD4 T cells per HFTOC sample. Error bars indicate standard deviations. *, $P < 0.05$.

genic HIV-1 Env encodes multiple, pathogenic determinants, including separable IFN induction activity and high fusion activity (14, 17, 22). As an *in vitro* model, the HFTOC has its limitation of generalizing the findings in other lymphoid organs and in an immunocompetent host. It will be important to further study the relative pathogenicity and IFN induction by R3A, R3B, and the chimeric recombinants in the humanized mouse models with a functional human immune system (24). We will also extend the findings in HIV-infected patients and in SIV-infected monkeys in future studies.

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REFERENCES

1. **Bosinger, S. E., et al.** 2009. Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. J. Clin. Invest. **119:**3556–3572.
2. **Duus, K. M., E. D. Miller, J. A. Smith, G. I. Kovalev, and L. Su.** 2001. Separation of human immunodeficiency virus type 1 replication from nef-mediated pathogenesis in the human thymus. J. Virol. **75:**3916–3924.
3. **Giorgi, J. V., et al.** 1993. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J. Acquir. Immune Defic. Syndr. **6:**904–912.
4. **Gurney, K. B., A. D. Colantonio, B. Blom, H. Spits, and C. H. Uittenbogaart.** 2004. Endogenous IFN-alpha production by plasmacytoid dendritic cells exerts an antiviral effect on thymic HIV-1 infection. J. Immunol. **173:**7269–7276.
5. **Hardy, A. W., D. R. Graham, G. M. Shearer, and J. P. Herbeuval.** 2007. HIV turns plasmacytoid dendritic cells (pDC) into TRAIL-expressing killer pDC and down-regulates HIV coreceptors by Toll-like receptor 7-induced IFN-alpha. Proc. Natl. Acad. Sci. U. S. A. **104:**17453–17458.
6. **Herbeuval, J. P., et al.** 2005. Regulation of TNF-related apoptosis-inducing ligand on primary CD4+ T cells by HIV-1: role of type I IFN-producing plasmacytoid dendritic cells. Proc. Natl. Acad. Sci. U. S. A. **102:**13974–13979.
7. **Herbeuval, J. P., et al.** 2006. Differential expression of IFN-alpha and TRAIL/DR5 in lymphoid tissue of progressor versus nonprogressor HIV-1-infected patients. Proc. Natl. Acad. Sci. U. S. A. **103:**7000–7005.
8. **Herbeuval, J. P., and G. M. Shearer.** 2007. HIV-1 immunopathogenesis: how good interferon turns bad. Clin. Immunol. **123:**121–128.
9. **Jacquelin, B., et al.** 2009. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. J. Clin. Invest. **119:**3544–3555.
10. **Katze, M. G., J. L. Fornek, R. E. Palermo, K. A. Walters, and M. J. Korth.** 2008. Innate immune modulation by RNA viruses: emerging insights from functional genomics. Nat. Rev. Immunol. **8:**644–654.
11. **Keir, M. E., C. A. Stoddart, V. Linquist-Stepps, M. E. Moreno, and J. M. McCune.** 2002. IFN-alpha secretion by type 2 predendritic cells up-regulates MHC class I in the HIV-1-infected thymus. J. Immunol. **168:**325–331.
12. **Kovalev, G., et al.** 1999. Induction of MHC class I expression on immature thymocytes in HIV-1-infected SCID-hu Thy/Liv mice: evidence of indirect mechanisms. J. Immunol. **162:**7555–7562.
13. **McCune, J. M.** 1997. Thymic function in HIV-1 disease. Semin. Immunol. **9:**397–404.
14. **Meissner, E. G., V. M. Coffield, and L. Su.** 2005. Thymic pathogenicity of an HIV-1 envelope is associated with increased CXCR4 binding efficiency and V5-gp41-dependent activity, but not V1/V2-associated CD4 binding efficiency and viral entry. Virology **336:**184–197.
15. **Meissner, E. G., K. M. Duus, F. Gao, X. F. Yu, and L. Su.** 2004. Characterization of a thymus-tropic HIV-1 isolate from a rapid progressor: role of the envelope. Virology **328:**74–88.
16. **Meissner, E. G., K. M. Duus, R. Loomis, R. D'Agostin, and L. Su.** 2003. HIV-1 replication and pathogenesis in the human thymus. Curr. HIV Res. **1:**275–285.
17. **Meissner, E. G., L. Zhang, S. Jiang, and L. Su.** 2006. Fusion-induced apoptosis contributes to thymocyte depletion by a pathogenic human immunodeficiency virus type 1 envelope in the human thymus. J. Virol. **80:**11019–11030.
18. **Miller, E. D., et al.** 2001. Human immunodeficiency virus type 1 IIIB selected for replication in vivo exhibits increased envelope glycoproteins in virions

without alteration in coreceptor usage: separation of in vivo replication from macrophage tropism. J. Virol. **75:**8498–8506.
19. **Poli, G., P. Biswas, and A. S. Fauci.** 1994. Interferons in the pathogenesis and treatment of human immunodeficiency virus infection. Antiviral Res. **24:** 221–233.
20. **Schnittman, S. M., S. Vogel, M. Baseler, H. C. Lane, and R. T. Davey, Jr.** 1994. A phase I study of interferon-alpha 2b in combination with interleukin-2 in patients with human immunodeficiency virus infection. J. Infect. Dis. **169:**981–989.
21. **Sedaghat, A. R., et al.** 2008. Chronic CD4+ T-cell activation and depletion in human immunodeficiency virus type 1 infection: type I interferon-mediated disruption of T-cell dynamics. J. Virol. **82:**1870–1883.
22. **Sivaraman, V., L. Zhang, E. G. Meissner, J. L. Jeffrey, and L. Su.** 2009. The heptad repeat 2 domain is a major determinant for enhanced HIV-1 fusion and pathogenicity of a highly pathogenic HIV-1 Env. J. Virol. **83:**11715–11725.
23. **Sodora, D. L., and G. Silvestri.** 2008. Immune activation and AIDS pathogenesis. AIDS **22:**439–446.
24. **Zhang, L., G. I. Kovalev, and L. Su.** 2007. HIV-1 infection and pathogenesis in a novel humanized mouse model. Blood **109:**2978–2981.